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Leptin attenuates the detrimental effects of β-amyloid on spatial memory and hippocampal later-phase long term potentiation in rats

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ABSTRACT

β-Amyloid (Aβ) is the main component of amyloid plaques developed in the brain of patients with Alzheimer's disease (AD). The increasing burden of Aβ in the cortex and hippocampus is closely correlated with memory loss and cognition deficits in AD. Recently, leptin, a 16 kD peptide derived mainly from white adipocyte tissue, has been appreciated for its neuroprotective function, although less is known about the effects of leptin on spatial memory and synaptic plasticity. The present study investigated the neuroprotective effects of leptin against Aβ-induced deficits in spatial memory and in vivo hippocampal late-phase long-term potentiation (L-LTP) in rats. Y maze spontaneous alternation was used to assess short term working memory, and the Morris water maze task was used to assess long term reference memory. Hippocampal field potential recordings were performed to observe changes in L-LTP. We found that chronically intracerebroventricular injection of leptin (1 μg) effectively alleviated Aβ1–42 (20 μg)-induced spatial memory impairments of Y maze spontaneous alternation and Morris water maze. In addition, chronic administration of leptin also reversed Aβ1–42-induced suppression of in vivo hippocampal L-LTP in rats. Together, these results suggest that chronic leptin treatments reversed Aβ-induced deficits in learning and memory and the maintenance of L-LTP.

Introduction

Alzheimer's disease (AD) is a progressively neurodegenerative disorder afflicting more than 40 million people all around the world. Its main pathological hallmarks include amyloid plaques consisting of extracellular β-amyloid (Aβ) and neurofibrillary tangles containing hyperphosphorylated tau in neurons. Although the exact pathogenesis of AD is still unknown, Aβ cascade hypothesis posits that mutation of several genes such as amyloid precursor protein (*APP*), presenilin-1 (*PS1*) and presenilin-2 (*PS2*), results in the neurotoxic Aβ production from amyloid precursor protein (APP) by β- and γ-secretases (Selkoe, 2002). Dysfunction of spatial memory including navigation deficits and memory retrieval disruption appeared at the early stage of AD patients and rodent models (Stuchlik et al., 2014). Direct injection of Aβ into the hippocampus or lateral ventricles in rats also led to a serious impairment in spatial cognition performance (Yamada et al., 1999).

Leptin is a 16 kD hormone secreted mainly from white adipocyte tissue. The plasma concentration of leptin is positively correlated with fat mass. Since identified in 1994 by Zhang et al. (1994), leptin has been known to regulate many physiological functions such as food intake, energy homeostasis, bone formation, reproduction, immunity, insulin sensitivity and neural activity (Tezapsidis et al., 2009). In recent years, the neuroprotective effects of leptin in AD, Parkinson disease, epilepsy, ischemia and glaucoma attracted the interests of neuroscientists (Folch et al., 2012; Komori et al., 2006; Marwarha and Ghribi, 2012; Valerio et al., 2009). For example, leptin prevents hippocampal synaptic disruption and neuronal cell death induced by Aβ (Doherty et al., 2013); leptin shows beneficial effects on the memory of transgenic mouse in novel object recognition test, contextual cued test and T maze foot shock avoidance (Farr et al., 2006; Greco et al., 2010).

In the present study, we investigated whether chronic intracerebroventricular (i.c.v.) injections of leptin could reverse Aβ1–42-induced impairments in short term spatial working memory and long term spatial reference memory by testing the spontaneous alternation in Y maze and the performance in Morris water maze. In addition, the hippocampal late-phase long-term potentiation (L-LTP), an important electrophysiological model for long term memory, was compared. We

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provide evidence that leptin alleviates Aβ1–42-induced impairments in learning, memory and the maintenance of L-LTP.

Materials and methods

Chemicals

Aβ1–42 (Abcam, UK) was prepared using procedures described previously (Doherty et al., 2013). Briefly, Aβ1–42 was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and then kept in room temperature with an occasional vortex for an hour. Next, the solution was sonicated for 10 min then aliquoted into smaller volumes and dried with nitrogen gas and stored at −80 °C. DMSO was used to resuspend the peptide and D-PBS was added before use. Leptin (Peprotec, USA) was dissolved in saline then aliquoted into smaller volumes and stored at −20 °C.

Animals and drug administration

Sprague–Dawley (SD) rats weighing between 250–300 g were used in the study. Food and water were available ad libitum. All animal handling and procedures accorded with the guidelines of the Shanxi Animal Research Ethics Committee. After 3 days of acclimation, rats were anesthetized with chloral hydrate (0.3 g/kg, i.p.) and positioned on a stereotaxic apparatus (RWD, Shenzhen, China). A cannula was implanted into the lateral ventricle and was fixed with dental cement. The coordinates of the cannula tip are 1.0 mm posterior, 1.5 mm lateral and 4.0 mm depth from bregma. After 5–7 days of recovery, Aβ1–42 (20 μg) was injected through the cannula into the ventricle. 24 h later, leptin (1 μg) was given through the cannula every day for 10 consecutive days. No drug injection was made during the period of behavioral tests (see below).

Behavioral tests

Y maze

Spontaneous alternation in the Y maze was tested after 10 days of drug administration. The Y maze has three arms separate from each other at the same angle, and each arm is 45 cm long. Rats were put into the end of a random arm of the Y maze and allowed to move freely for an 8 min session. The entries of rats into each arm were recorded and every entry different from last two entries was considered as a successful alternation. The alternation percentage was calculated according to the following: [(number of alternations) / (total number of arm entries − 2)] × 100(%) (Iwai et al., 2014).

Morris water maze

The Morris water maze test was carried out in a circular tank (150 cm diameter) filled with tap water. The temperature of the water was maintained at 22–25 °C. A camera hanging above the tank was used to record swimming traces of rats. The pool was divided into four quadrants and a hidden platform was set 1–2 cm under water in the targeted quadrant. Each rat was placed in the water facing the wall of the tank from a different quadrant in all trials. The learning ability of rats was examined using the hidden platform test from the 1st to the 5th days, which allowed rats to swim 4 times per training day. Memory test was evaluated by 2 times of probe trials on the 6th day after removing the platform out of the pool. Visual and locomotor abilities of rats were examined using the visual platform test. The escape latency in the hidden platform test and swimming traces in all experiments were recorded for off-line analysis with software (Ethovision 3.0, Noldus Information Technology, Wageningen, Netherlands).

In vivo hippocampal L-LTP recording

The following day after the Morris water maze test, rats were anesthetized with urethane (0.3 g/kg, intraperitoneal) and positioned on a stereotaxic apparatus. A hole with an approximate 2.0 mm diameter was drilled on the skull (3.0 mm posterior from bregma and 4.2 mm from midline). A concentric stimulating electrode (FHC, USA) and a recording electrode were inserted into the CA1 region of the hippocampus to record field excitatory postsynaptic potentials (fEPSPs) in stratum radiatum by stimulating the Schaffer collateral/associational commissural pathway. Basal fEPSPs were first recorded for 30 min with test stimulation (intensity, 30–50% of maximal EPSPs; frequency, 0.033 Hz). High-frequency stimulation (HFS) was applied to induce L-LTP of fEPSP. The HFS consisted of 3 series with a 5 min interval, each series containing 3 trains of 20 stimuli with an inter-stimulus interval of 5 ms (200 Hz) and an inter-train interval of 30 s, as described in our previous studies (Han et al., 2013; Qiao et al., 2014). After 3 series of HFS, fEPSPs were recorded at 0.033 Hz for at least 3 h. The amplitude of fEPSPs was normalized to basal fEPSPs and averaged. To test if presynaptic mechanism was involved, two paired stimuli with an interval of 50 ms were also applied during basal recordings of fEPSPs.

Data analysis

Data are shown as the mean ± standard error. Statistical analysis was conducted by using two-way ANOVA for field potential recording and behavioral tests; three repeated ANOVA was used in the Morris navigation test. All data were calculated using SPSS 18.0 package. Significance level was set at $P < 0.05$. Effect sizes were computed as η^2 for ANOVAs.

Results

A cannula was chronically implanted into the lateral ventricle for i.c.v. injection. Aβ1–42 (20 μg) was injected through the cannula into the ventricle. 24 h later, leptin (1 μg) was given through the cannula every day for 10 consecutive days. The behavioral and LTP experiments were carried out thereafter. The timeline for the entire experiments are shown in Supplementary Fig. 1A. We found that leptin injections produced modest decreases in the body weight of rats compared with those of the two other groups (leptin effect: $F_{(1,39)} = 64.769$, $P < 0.001$, $\eta^2 = 0.141$, Supplementary Fig. 1B).

Leptin reversed Aβ1–42-induced impairment of spatial working memory

Spontaneous alternation of the Y maze was performed to examine the spatial working memory of rats. Two-way ANOVA showed that Aβ1–42 and leptin treatment had significant main effects on the spontaneous alternation of rats (Aβ1–42: $F_{(1,34)} = 19.774$, $P < 0.001$, $\eta^2 = 0.382$; leptin: $F_{(1,34)} = 4.316$, $P < 0.05$, $\eta^2 = 0.118$; Aβ1–42 by leptin interaction: $F_{(1,34)} = 9.628$, $P < 0.01$, $\eta^2 = 0.231$). As shown in Fig. 1A, Tukey's post-hoc tests showed that Aβ1–42 significantly decreased in the percentage of right alternation of rats ($P < 0.001$, n = 8–10) and leptin reversed this detrimental effect ($P < 0.001$, n = 9). At the same time, the total arm entries of rats did not show any significant difference among these groups ($P > 0.05$, $\eta^2 = 0.004$; Fig. 1B), suggesting that the difference in spontaneous alternation among these groups is due to the change in spatial working memory, but not to the impaired locomotor activity of the rats.

Leptin injection protected against Aβ1–42-induced impairment of spatial reference memory

The Morris water maze was used to test the spatial learning and memory of the rats. In consecutive 5 days of the hidden platform tests, the escape latency in all groups showed a gradual decrease with the increase of training days ($F_{(4,156)} = 173.395$, $P < 0.001$, $\eta^2 = 0.721$; Fig. 2A). Significant main effects existed in Aβ1–42 vs. vehicle treatment ($F_{(1,39)} = 9.656$, $P < 0.01$, $\eta^2 = 0.063$) and leptin vs. saline treatment ($F_{(1,39)} = 12.115$, $P < 0.01$, $\eta^2 = 0.071$) on escape latency. There was